Advanced NMR: Metabolite ID by NMR

UAB Metabolomics Training Course

June 14-18, 2015

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NIH Eastern Regional Comprehensive Metabolomics Resource Core
(RTI RCMRC)

Outline

- Information that NMR Spectroscopy data can provide
  - Chemical shift, J-coupling, chemical structure
- Available NMR methods
  - 1D NMR: ^{1}H, ^{13}C, ^{15}N, ^{31}P
  - 2D NMR: COSY, TOCSY, HSQC, HMBC, NOESY, INADEQUATE
  - Selective 1D: 1D TOCSY, 1D HSQC
- Spectral editing methods
  - CPMG, Diffusion, JRES, DEPT (DEPT 45, DEPT 90, DEPT 135)
- NMR Libraries, software, and databases
  - AMIX, BBREFCODE (Bruker), BATMAN, Chenomx, COLMAR, HMDB, BMRB, Birmingham Metabolite Library, NMR Shift DB
- Other complementary methods
  - Eg. STOCSY, STORM, RANSY
  - MUMA Package (R Based) for STOCSY and STORM
- Tagging, Spike-in of metabolites, Predicting Spectra

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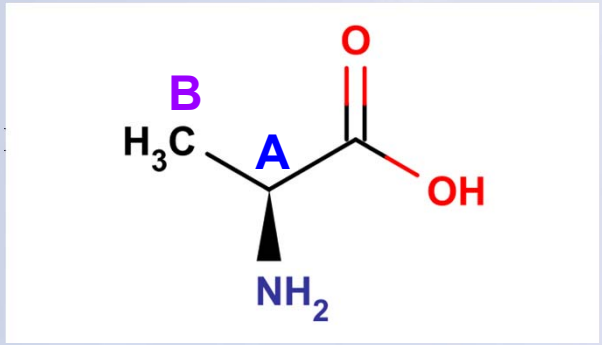
NMR Chemical Shift



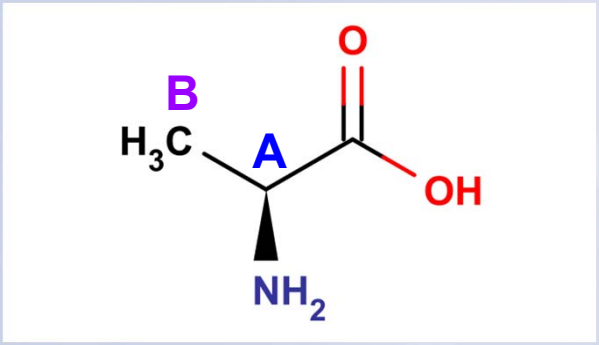
Alanine

- Peak position is called "chemical shift"
- It depends on the chemical environment
- Splitting of peak is dependent on neighboring ^1H atom(s)
- Area under peak proportional to the number of ^1H atoms underlying it

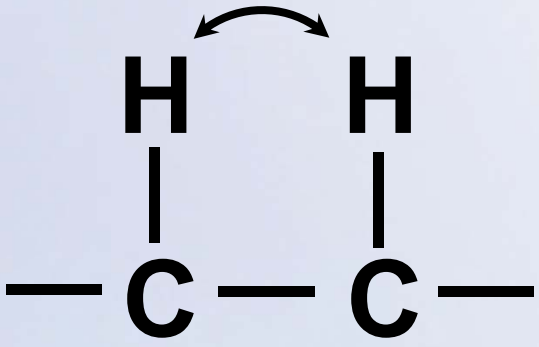
Chemical shift of C**H**
4 lines (quadrat)

Chemical shift of C**H_3**
2 lines (doublet)

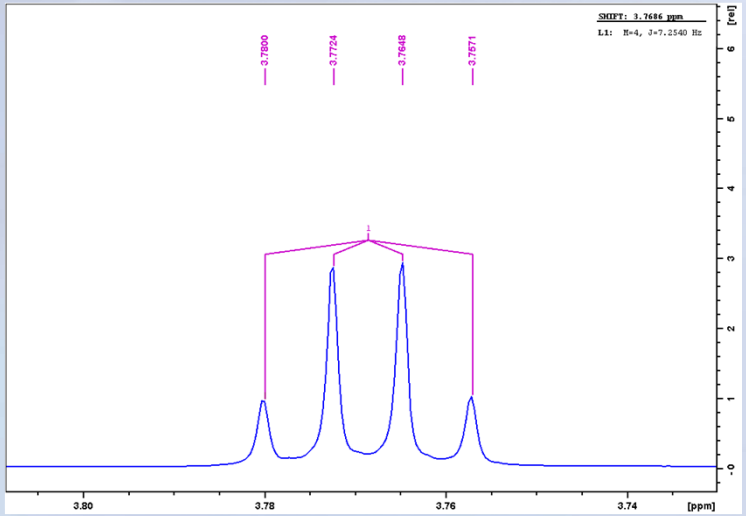
NMR J-Coupling



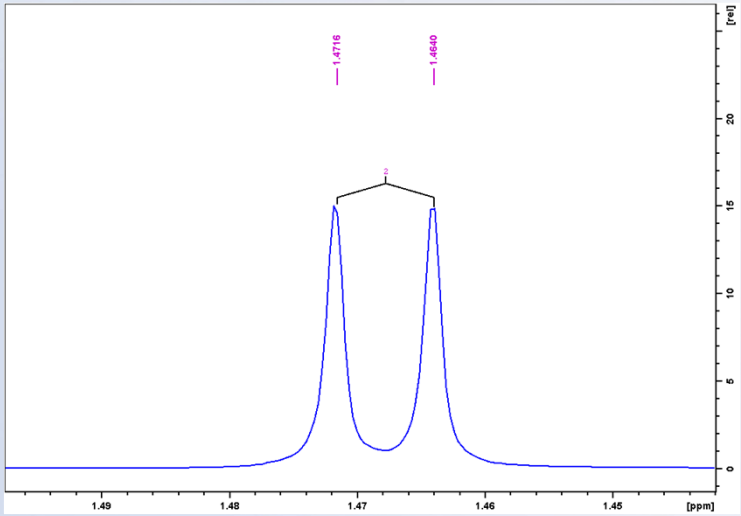
Alanine



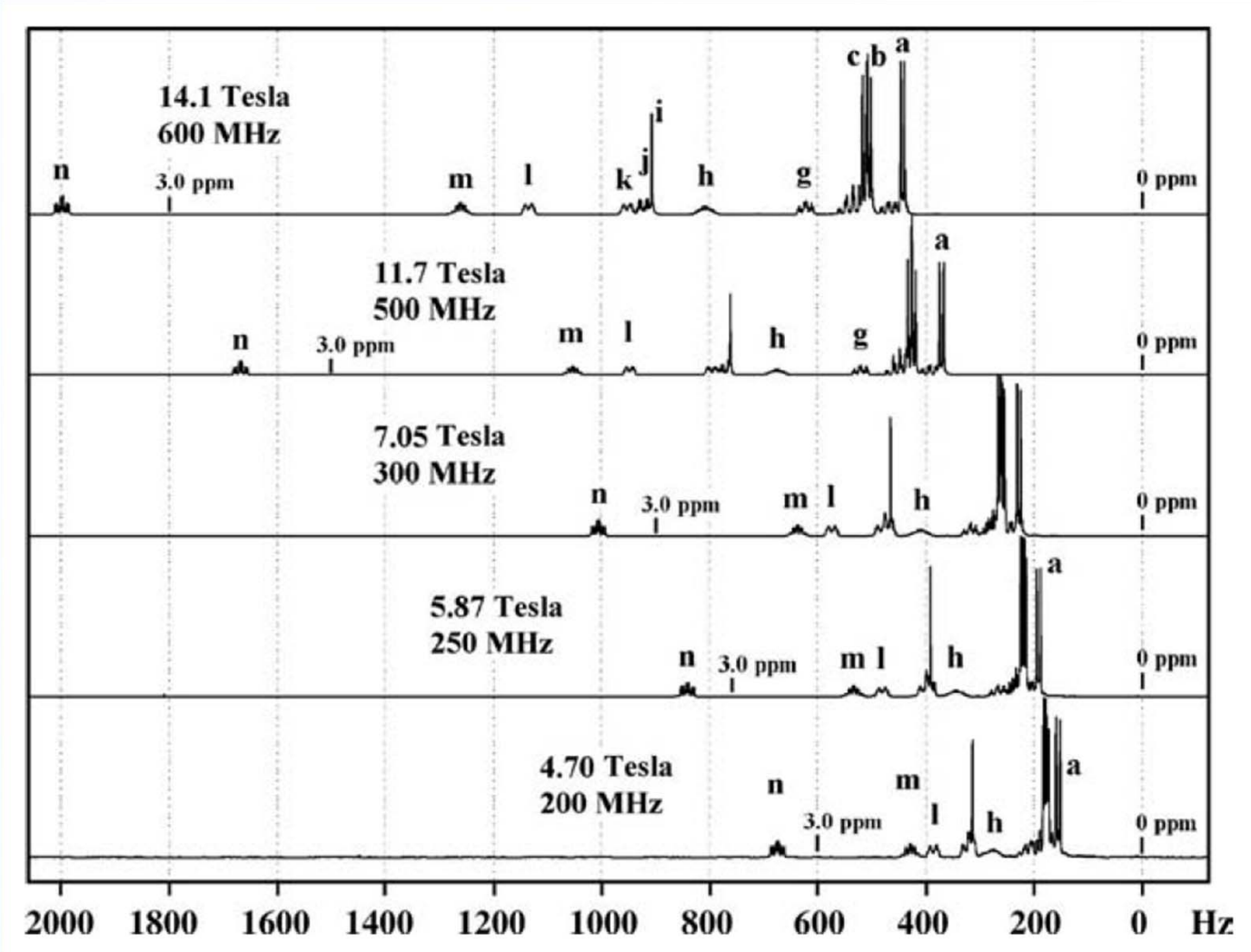
J = 7.2 Hz



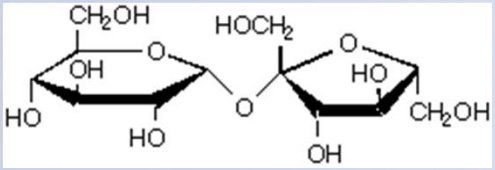
J = 7.2 Hz



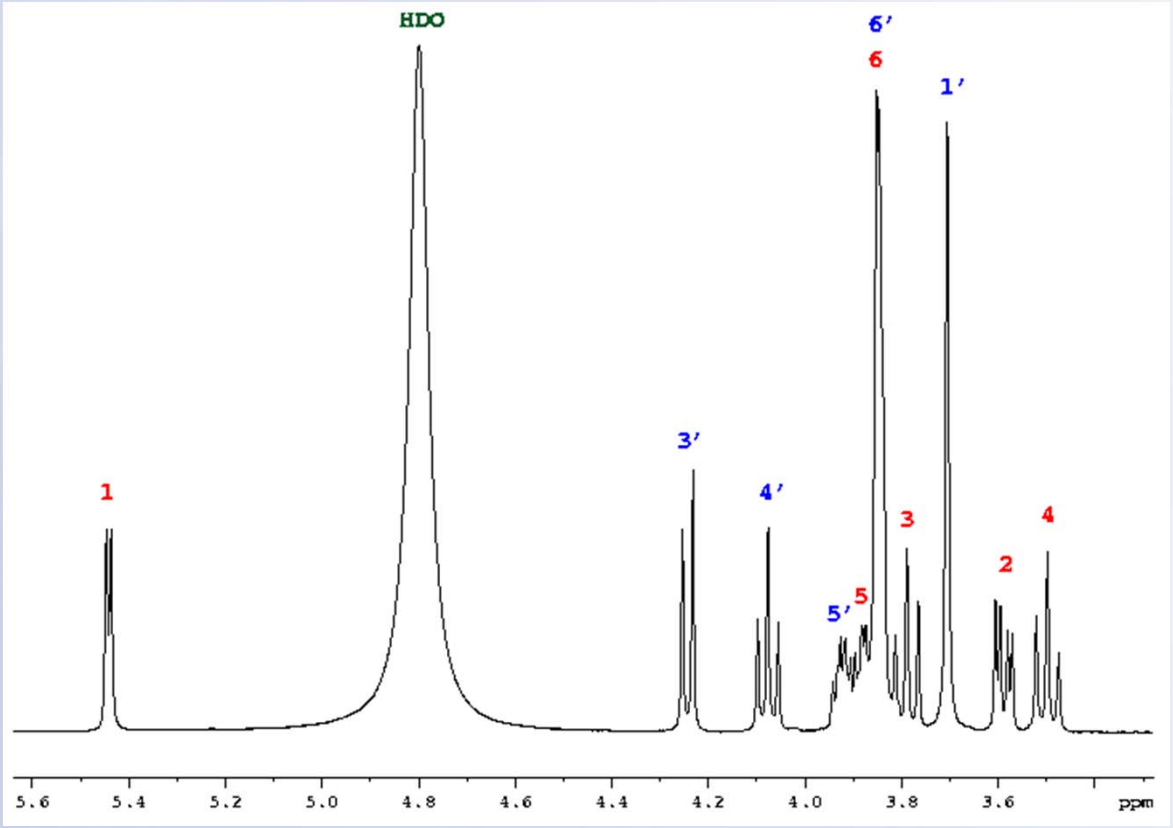
Dispersion of NMR Signal with Magnetic Field Strength



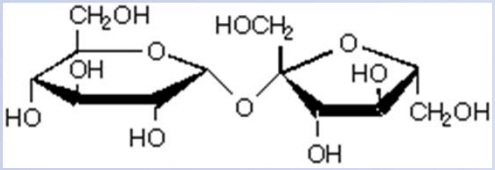
1D and 2D NMR Methods



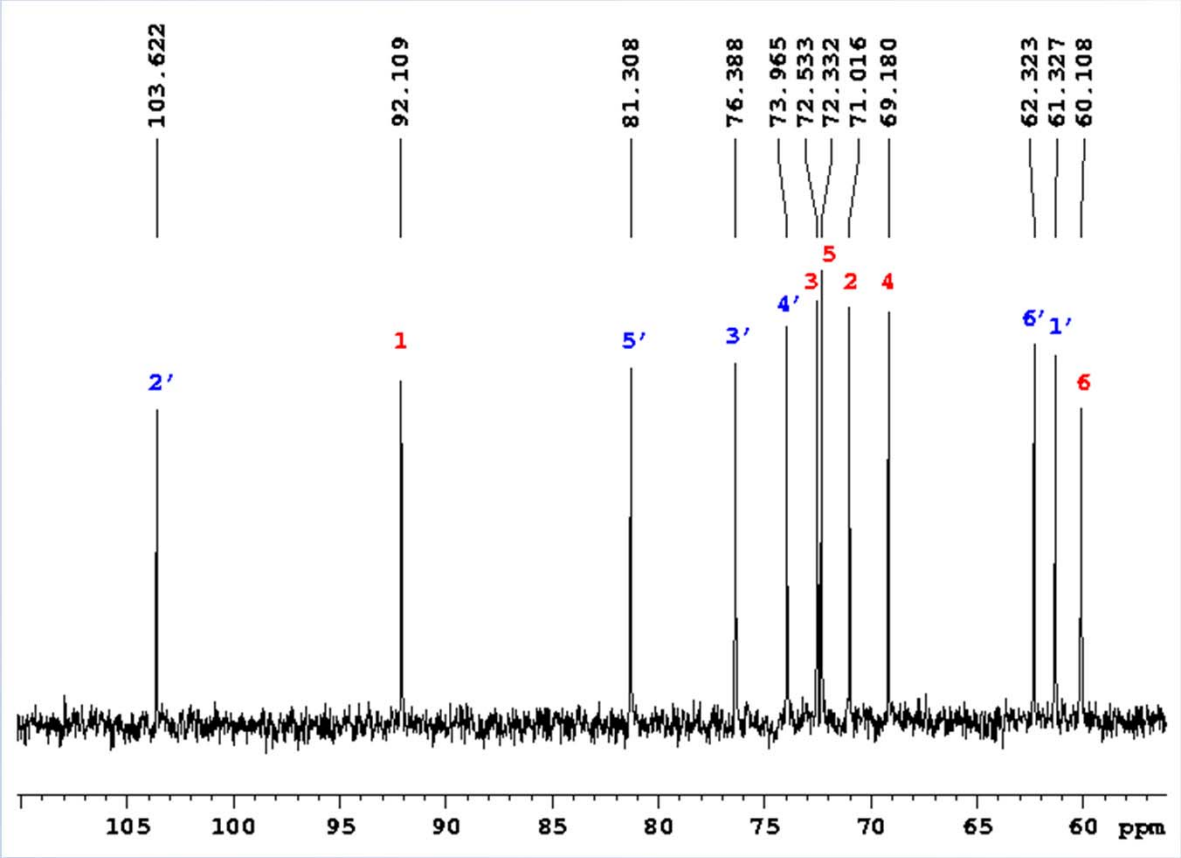
^{1}H NMR: Sucrose



NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

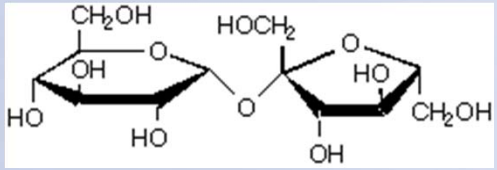


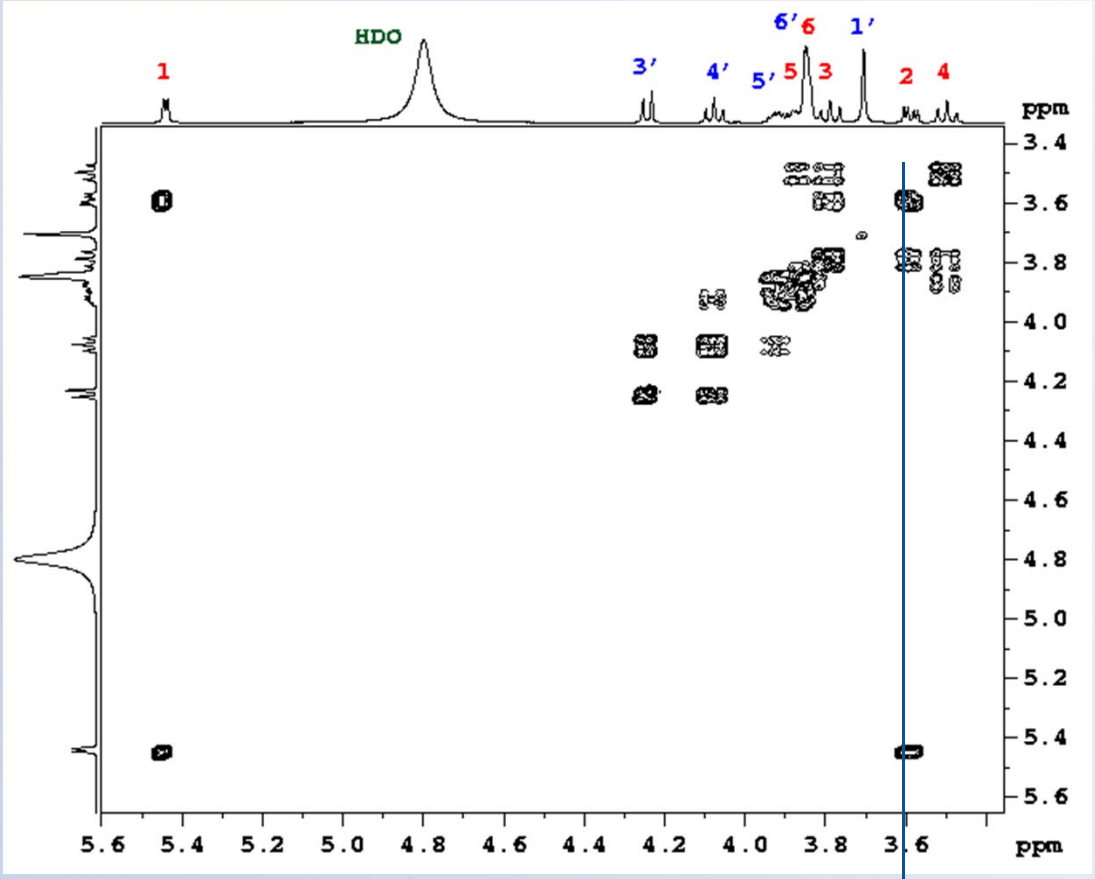
^{13}C NMR: Sucrose



NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

COSY: Sucrose





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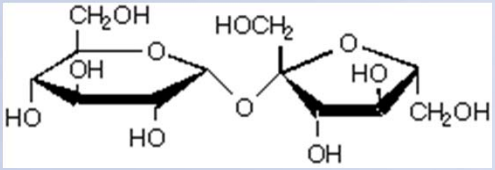
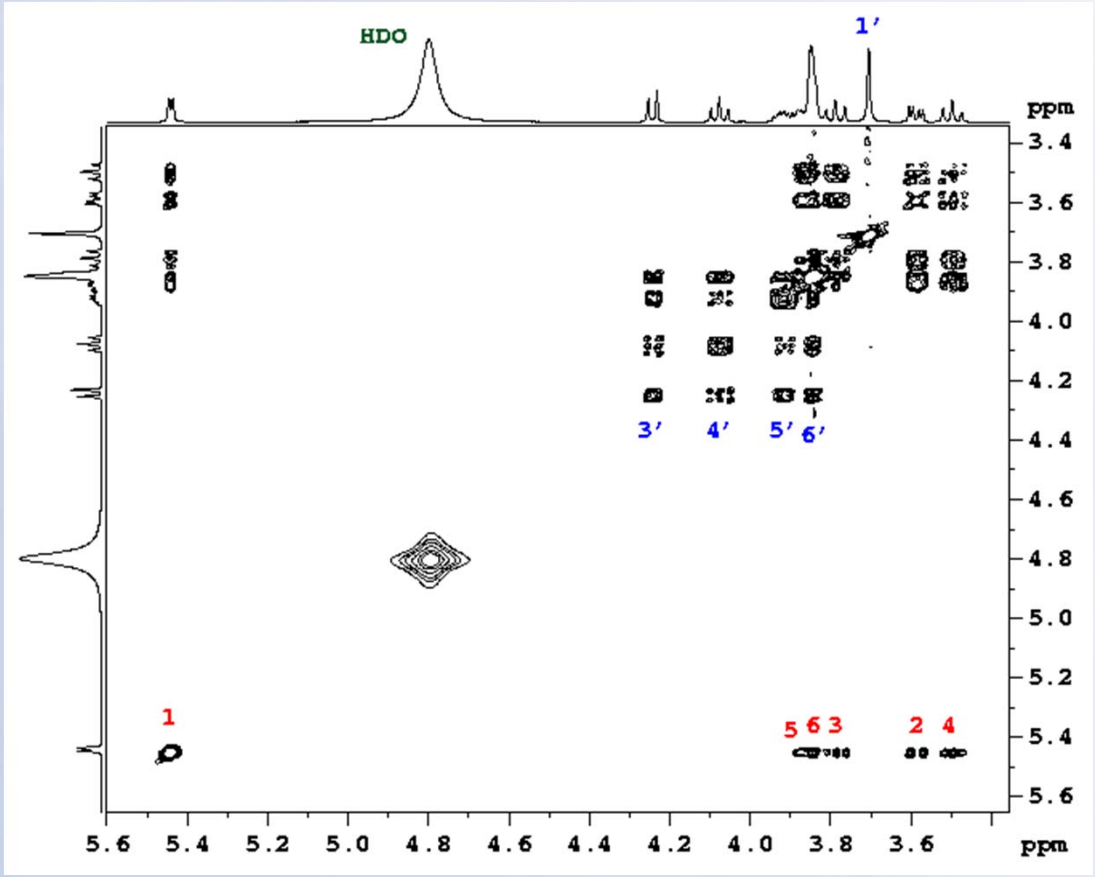
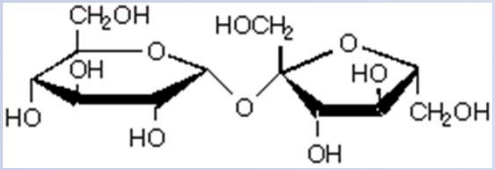


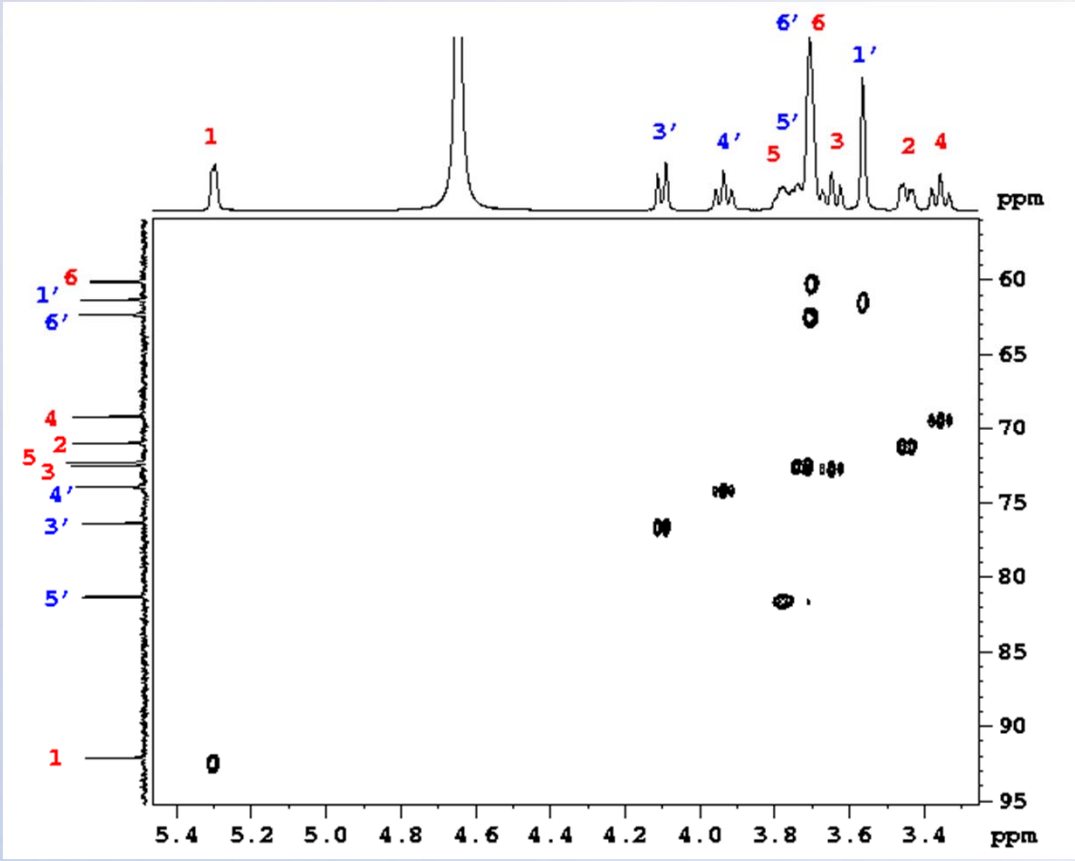
TOCSY: Sucrose



NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

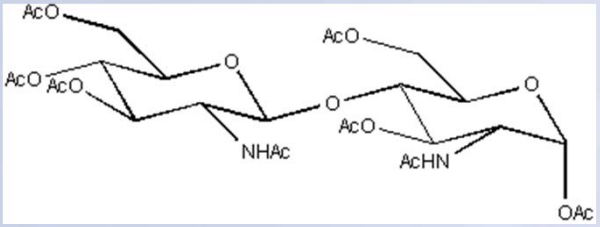
HSQC: Sucrose

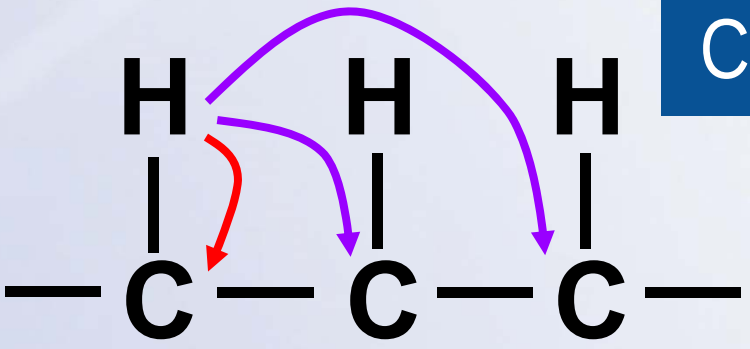


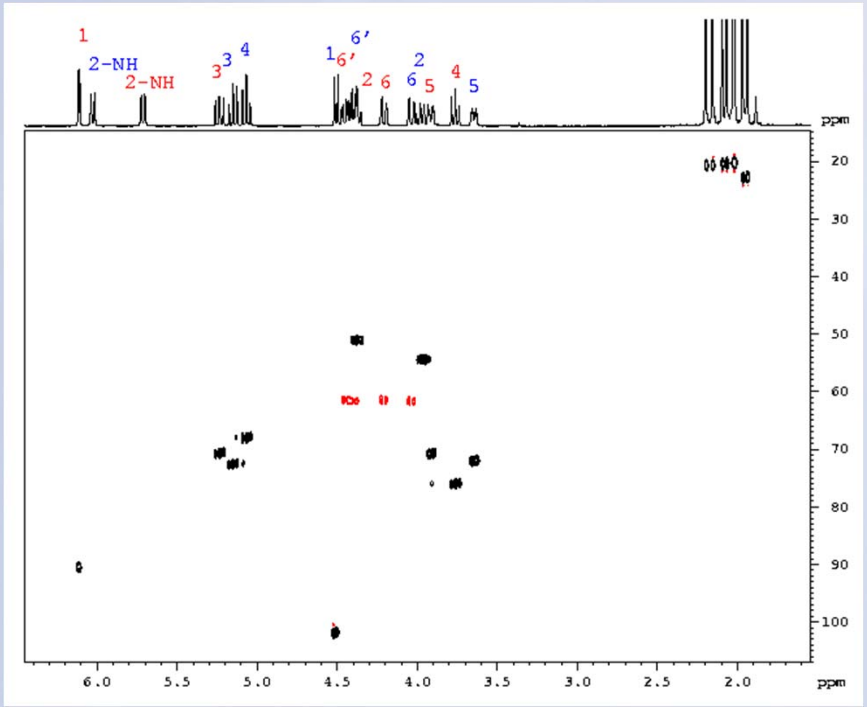


NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

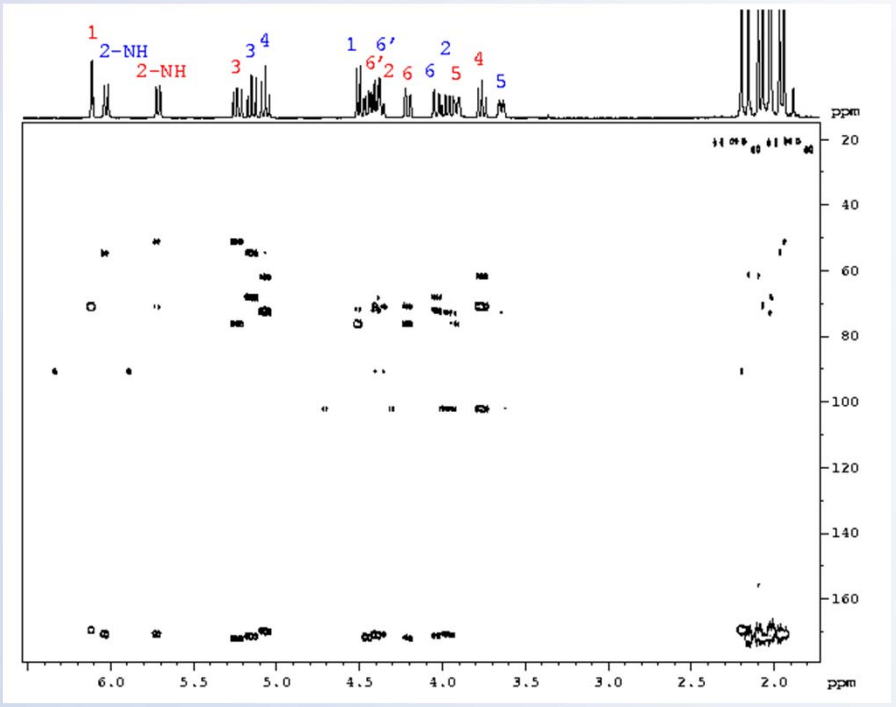
Chentobiose







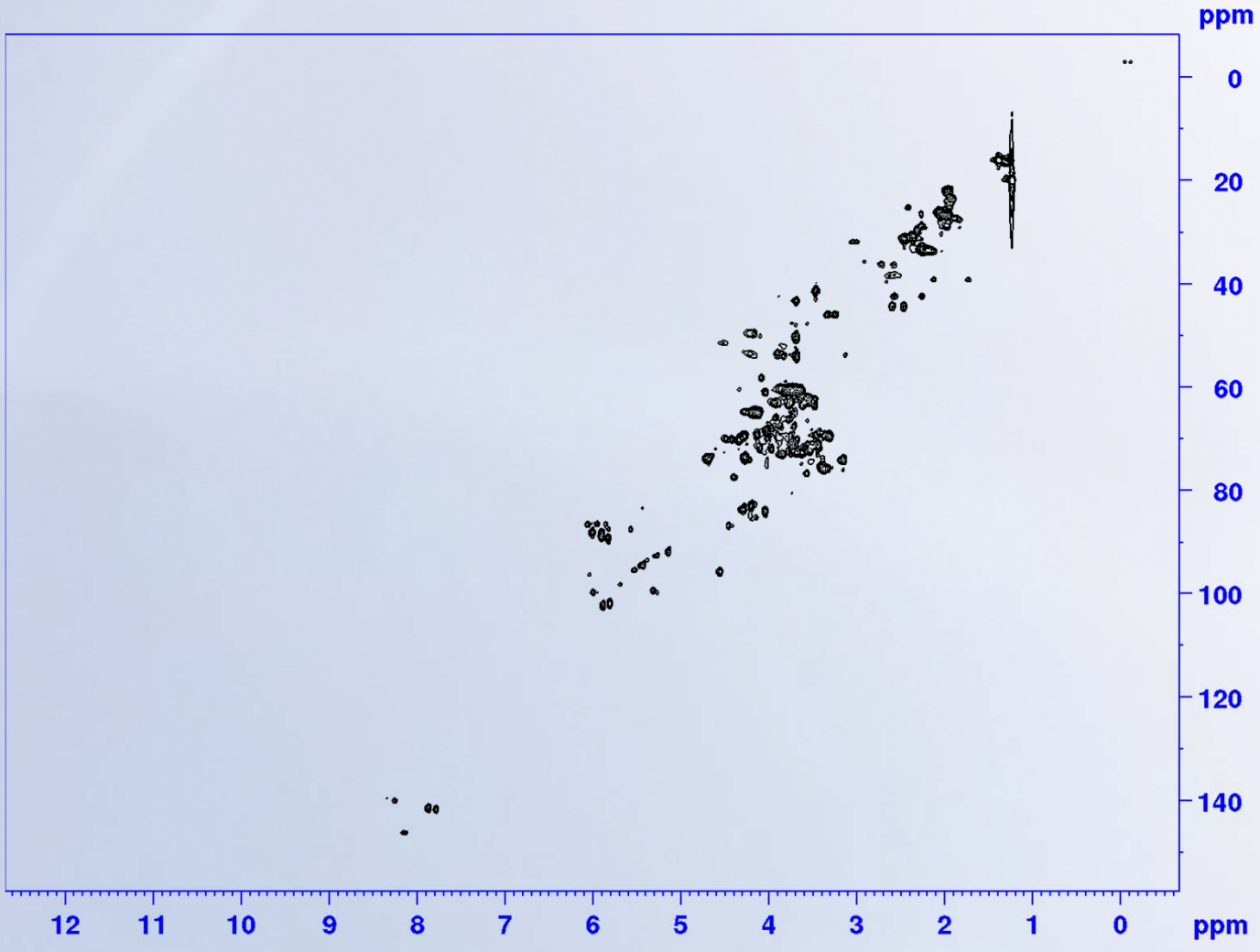
HSQC ($^{1}J_{CH}$)



HMBC ($^{2}J_{CH}$, $^{3}J_{CH}$)

NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

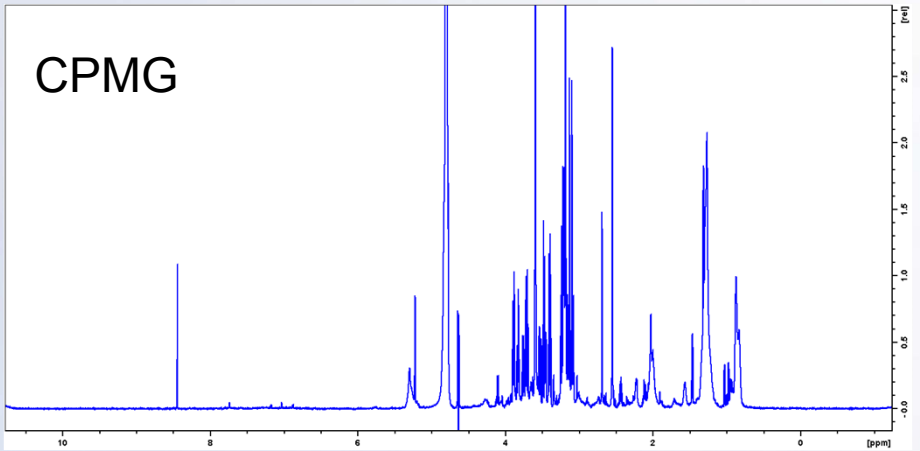
^{1}H-^{13}C HSQC Spectrum of Cell Extract

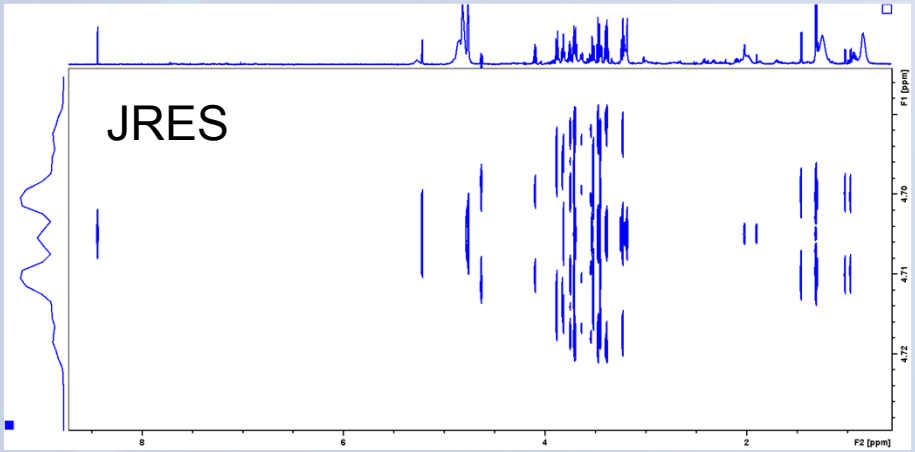


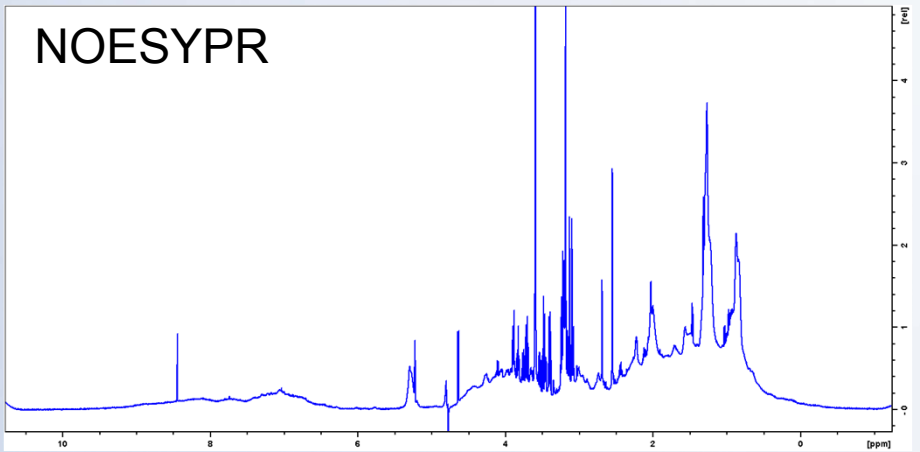
Spectral Editing

Spectral Editing

- Relaxation editing
  - CPMG Pulse sequence
- J- Modulated
  - J-RES

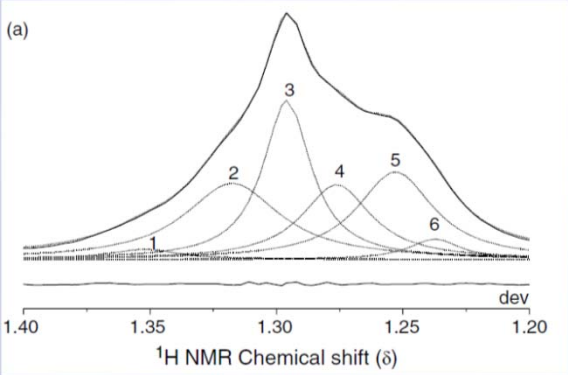


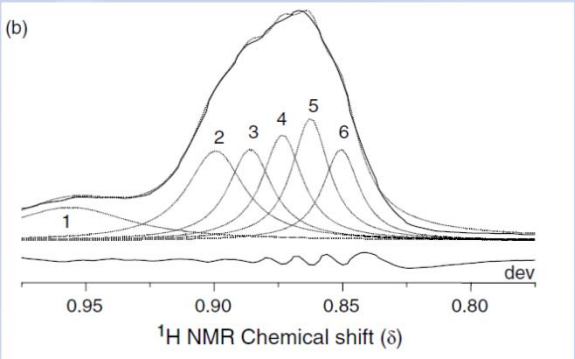


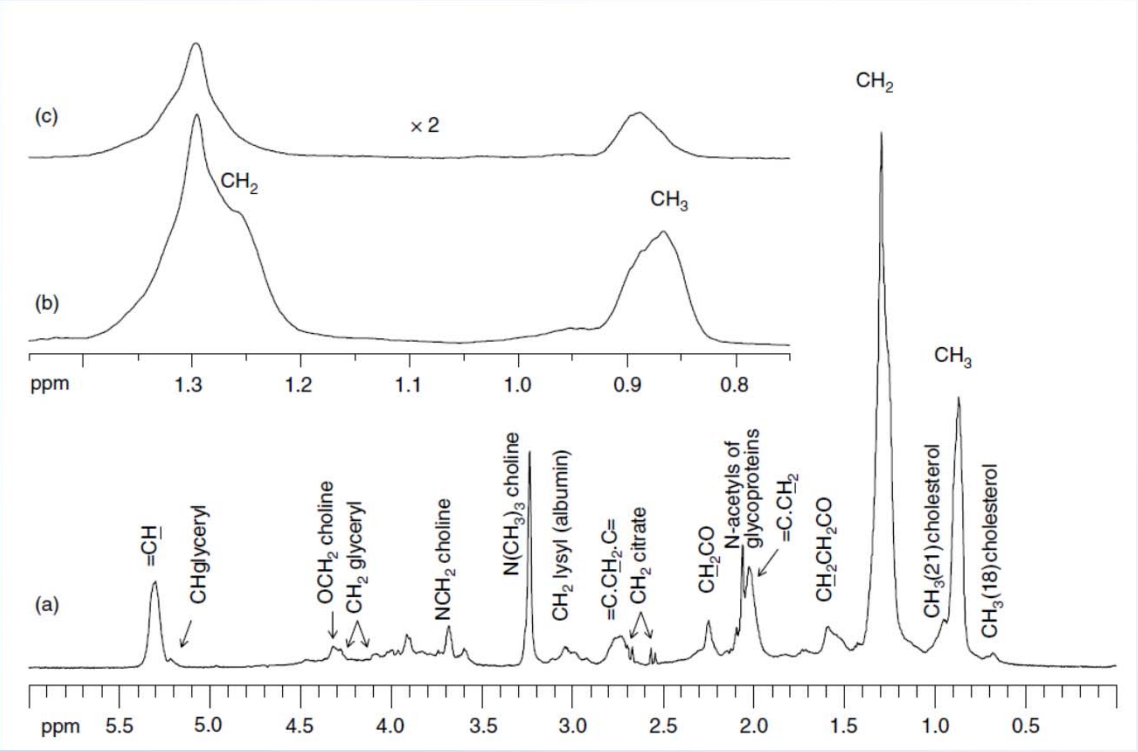


Analysis of Lipoproteins

- Lipoproteins are classified based on their size and density (VLDL, LDL, HDL)
- Lipoproteins can be assigned to these sub fractions by deconvoluting the CH_3 and $(CH_2)_n$ in diffusion edited spectra



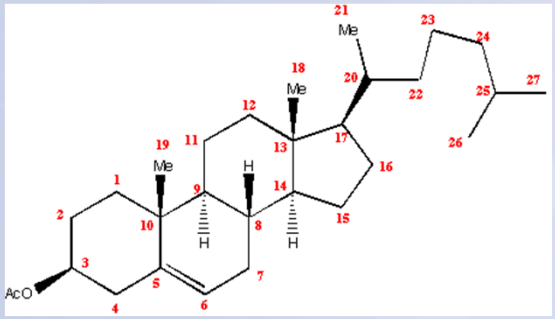


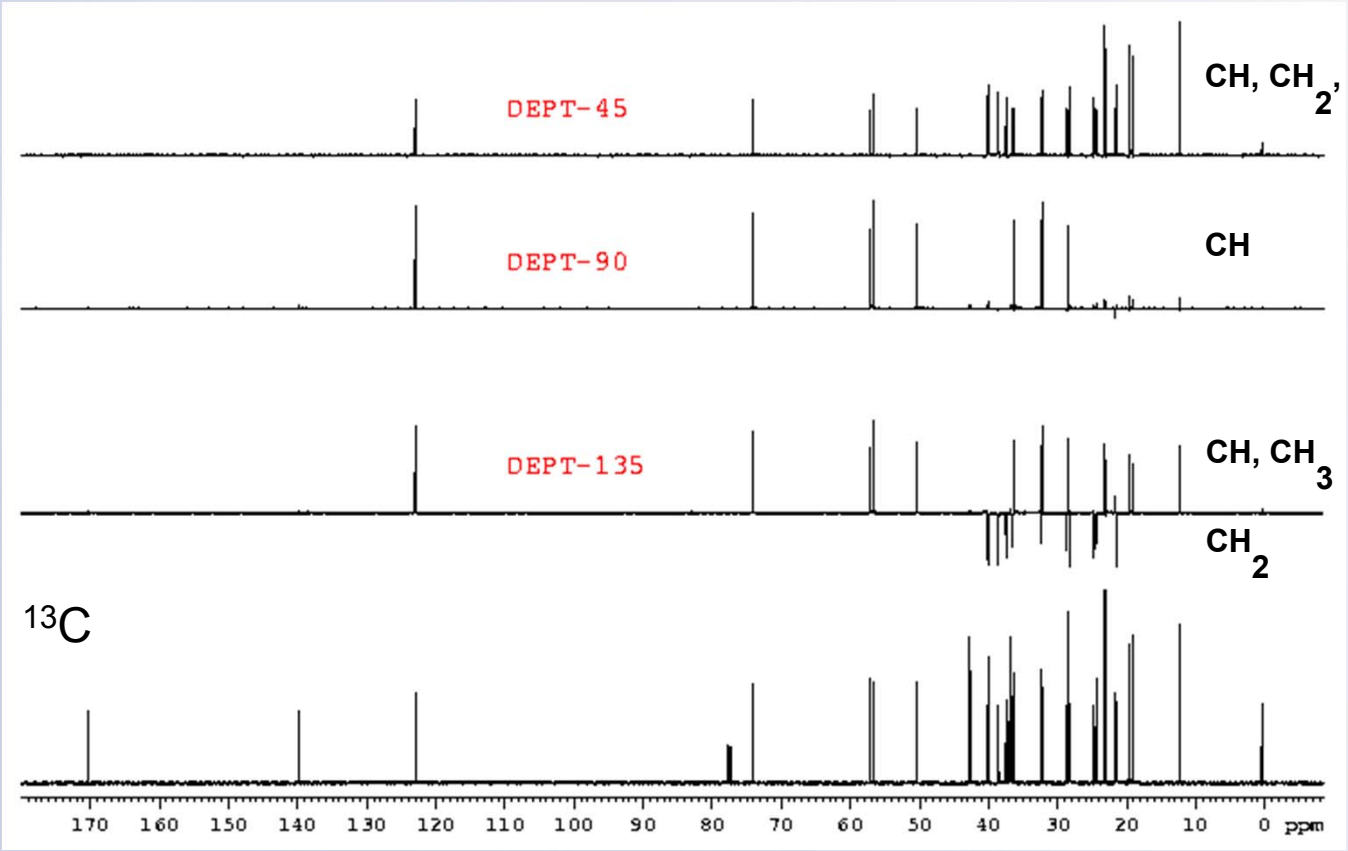


Diffusion Edited NMR Spectra

Peak	δ (ppm)	Width (Hz)	D ($cm^2\ s^{-1}$ $\times 10^7$)[a]	Area (%)[b]	Assignment
$(CH_2)_n$ 1	1.353	19.7	2.72	2.3	LDL + VLDL
$(CH_2)_n$ 2	1.317	28.0	1.98	26.4	VLDL
$(CH_2)_n$ 3	1.296	14.0	1.85	27.9	VLDL
$(CH_2)_n$ 4	1.276	17.8	3.15	15.5	LDL
$(CH_2)_n$ 5	1.255	20.5	5.19	19.9	HDL(60.6%) + LDL(39.4%)
$(CH_2)_n$ 6	1.240	18.4	5.96	7.9	HDL
CH_3 1	0.956	33.9	3.77	15.8	VLDL + HDL
CH_3 2	0.899	16.4	1.70	20.6	VLDL
CH_3 3	0.886	12.4	1.84	15.9	VLDL
CH_3 4	0.873	11.3	3.07	16.7	LDL
CH_3 5	0.862	10.5	4.51	17.9	HDL(40.8%) + LDL(59.2%)
CH_3 6	0.851	10.2	7.11	13.1	HDL

DEPT: Cholesterol





NMR Libraries, Software, and Databases

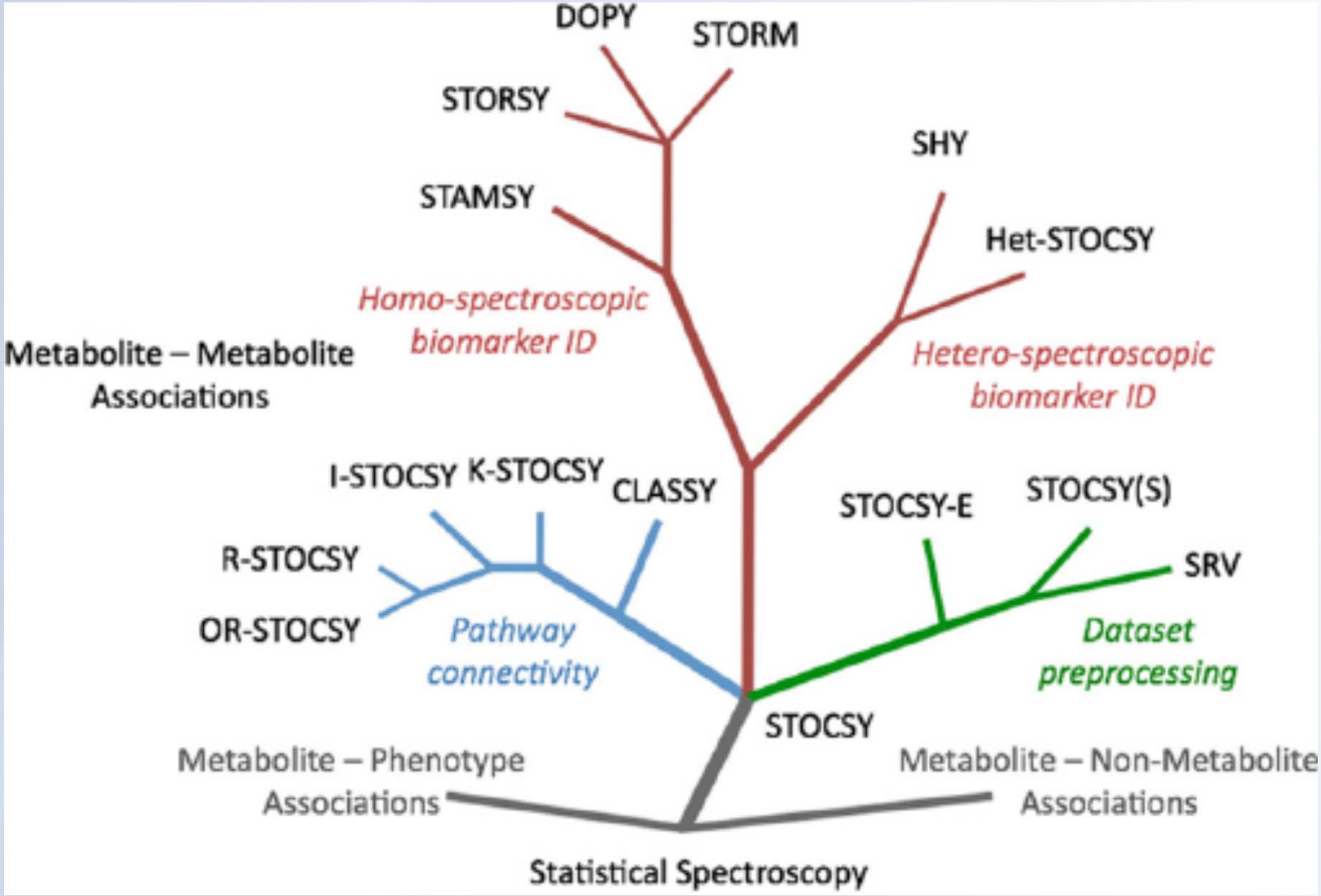
NMR Libraries and Databases

- Continuously emerging
- Databases
  - HMDB (http://www.hmdb.ca/)
  - Birmingham Metabolite Library (http://www.bml-nmr.org/)
  - BMRB (http://www.bmrb.wisc.edu/)
  - NMRShift DB (http://nmrshiftdb.nmr.uni-koeln.de/)
- Online Software
  - COLMAR (http://spin.ccic.ohio-state.edu/)
- Standalone Software
  - Chenomx (http://www.chenomx.com/
  - AMIX/ ASSURE/BBREFCODE

  https://www.bruker.com/products/mr/nmr/nmr-software/software/amix/overview.html
  - BATMAN (http://batman.r-forge.r-project.org/)
  - CCPN Metabolomics (http://www.ccpn.ac.uk/collaborations/metabolomics)
  - rNMR (link)

Other Complementary methods

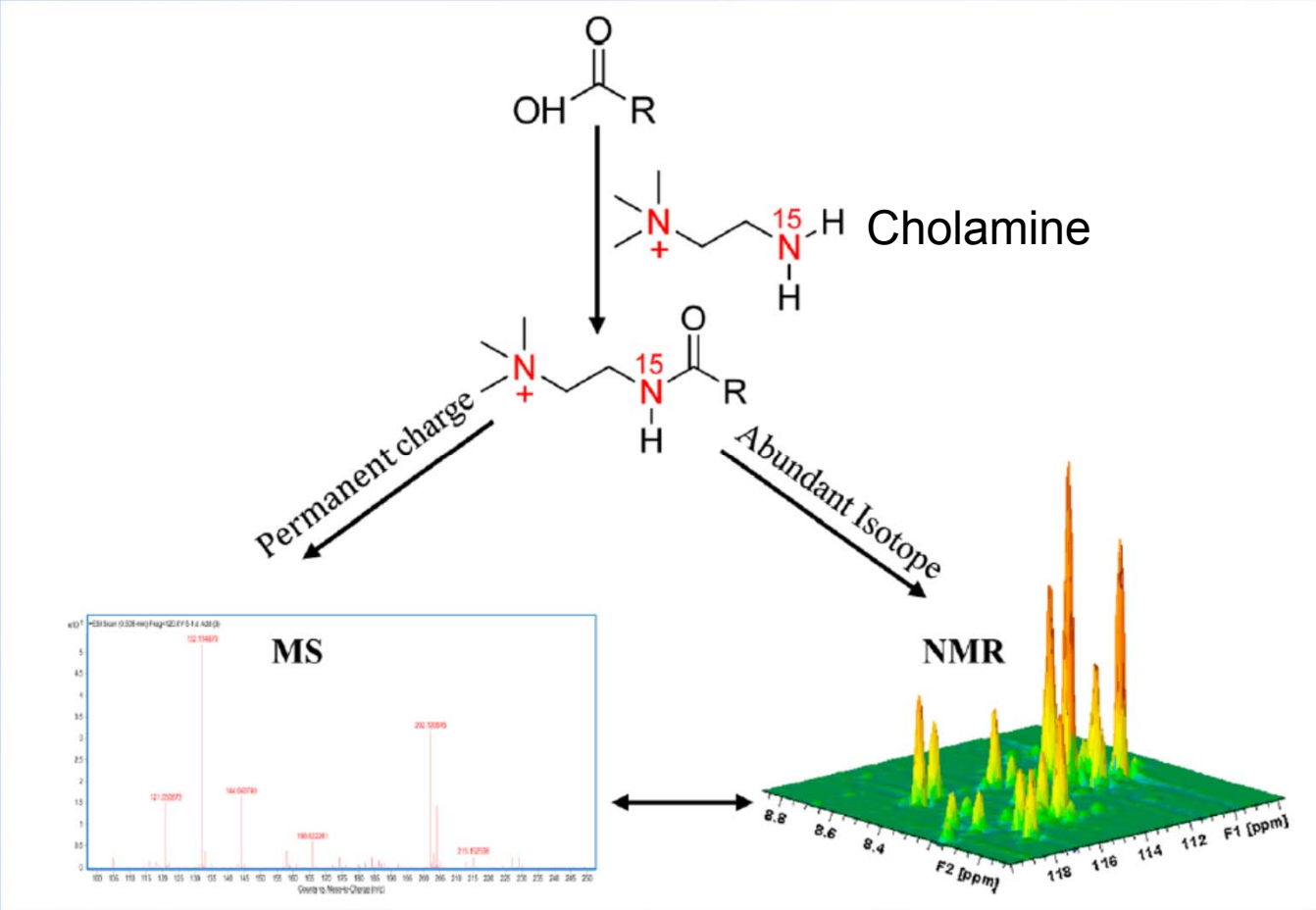
Statistical Spectroscopic Tools



Robinette, S. L., J. C. Lindon and J. K. Nicholson (2013). "Statistical spectroscopic tools for biomarker discovery and systems medicine." Anal Chem 85(11): 5297-5303.

Tagging

Smart Tagging

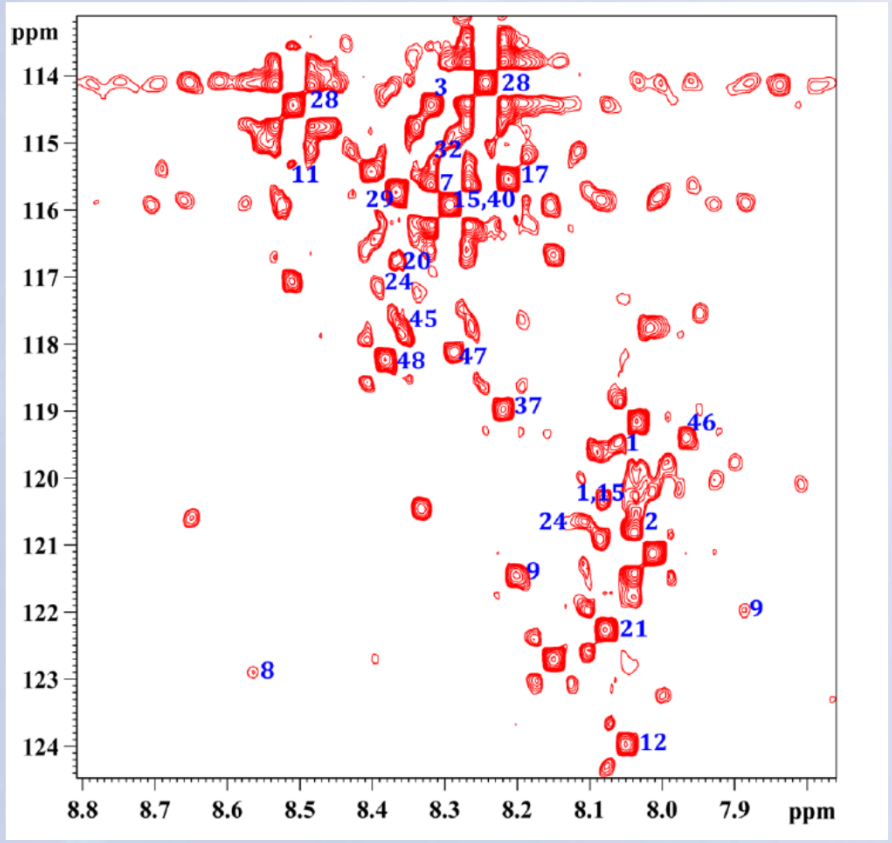


Tayyari, F., G. A. Gowda, H. Gu and D. Raftery (2013). "15N-cholamine--a smart isotope tag for combining NMR- and MS-based metabolite profiling." Anal Chem **85**(18): 8715-8721

Smart Tagging

^{1}H-^{15}N HSQC

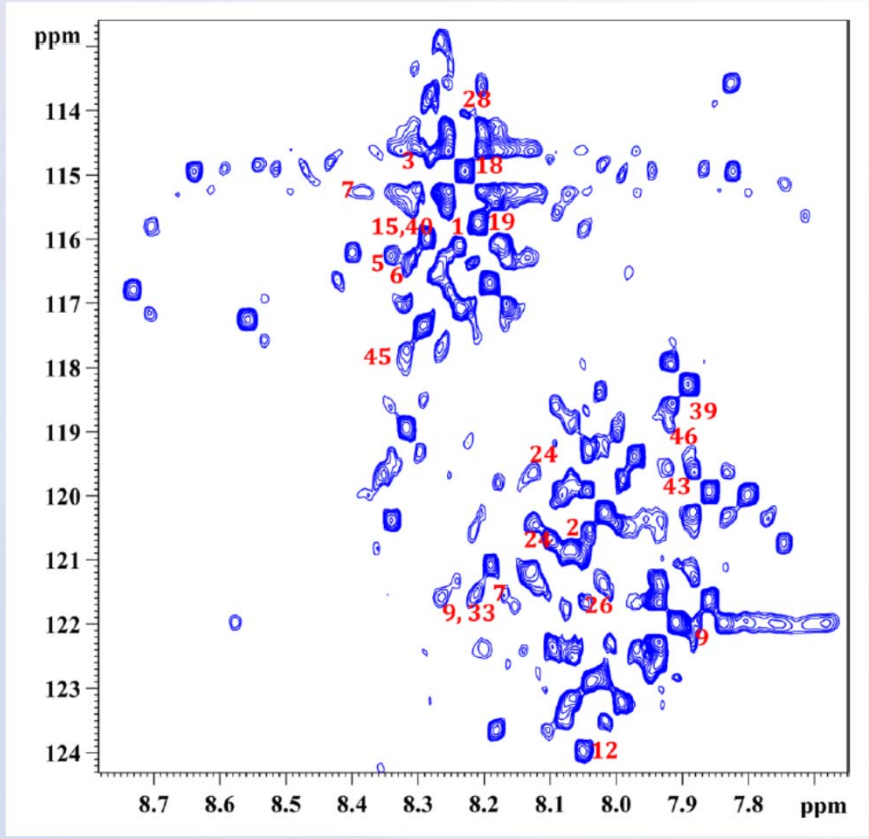
Human Serum



^{15}N

^{1}H

Human Urine



^{1}H

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References

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RTI INTERNATIONAL

Chenomx Exercise

Chenomx Library

- Over 320 metabolites
- pH sensitive library of 1H NMR Spectra
- Customizable

1,3-Dihydroxyacetone, 1,3-Dimethylurate, 1,6-Anhydro-β-D-glucose, 1,7-Dimethylxanthine, 1-Methylnicotinamide, 2'-Deoxyadenosine, 2'-Deoxyguanosine, 2'-Deoxyinosine, 2-Aminoadipate, 2-Aminobutyrate, 2-Ethylacrylate, 2-Furoate, 2-Hydroxy-3-methylvalerate, 2-Hydroxybutyrate, 2-Hydroxyglutarate, 2-Hydroxyisobutyrate, 2-Hydroxyisocaproate, 2-Hydroxyisovalerate, 2-Hydroxyphenylacetate, 2-Hydroxyvalerate, 2-Methylglutarate, 2-Octenoate, 2-Oxobutyrate, 2-Oxocaproate, 2-Oxoglutarate, 2-Oxoisocaproate, 2-Oxovalerate, 2-Phosphoglycerate, 3,4-Dihydroxymandelate, 3,5-Dibromotyrosine, 3-Aminoisobutyrate, 3-Chlorotyrosine, 3-Hydroxy-3-methylglutarate, 3-Hydroxybutyrate, 3-Hydroxyisovalerate, 3-Hydroxymandelate, 3-Hydroxyphenylacetate, 3-Indoxylsulfate, 3-Methyl-2-oxovalerate, 3-Methyladipate, 3-Methylxanthine, 3-Phenyllactate, 3-Phenylpropionate, 4-Aminobutyrate, 4-Aminohippurate, 4-Hydroxy-3-methoxymandelate, 4-Hydroxybenzoate, 4-Hydroxybutyrate, 4-Hydroxyhippurate, 4-Hydroxyphenyllactate, 4-Pyridoxate, 5,6-Dihydrothymine, 5,6-Dihydrouracil, 5-Aminolevulinate, 5-Hydroxyindole-3-acetate, 5-Hydroxylysine, 5-Methoxysalicylate, Acetaldehyde, Acetamide, Acetaminophen, Acetate, Acetoacetate, Acetone, Acetylsalicylate, Adenine, Adenosine, Adipate, Alanine, Allantoin, Alloisoleucine, Anserine, Arginine, Argininosuccinate, Asparagine, Aspartate, Benzoate, Betaine, Biotin, Butyrate, Butyrylcarnitine, Caffeine, Caprate, Caprylate, Carnitine, Carnosine, Choline, Cinnamate, Citrate, Citrulline, Creatine, Creatinine, Cysteine, Cystine, Cytidine, Cytosine, DSS (Chemical Shift Indicator), Dimethylamine, Epicatechin, Ethanol, Ethanolamine, Ethylene glycol, Ethylmalonate, Ferulate, Formate, Fructose, Fucose, Fumarate, Galactarate, Galactitol, Galactonate, Galactose, Gentisate, Glucarate, Glucose, Glutamate, Glutamine, Glutarate, Glutaric acid monomethyl ester, Glutathione, Glycerate, Glycerol, Glycine, Glycolate, Glycylproline, Guanidoacetate, Guanine, Hippurate, Histidine, Homocitrulline, Homocystine, Homogentisate, Homoserine, Homovanillate, Hypoxanthine, Ibuprofen, Imidazole, Indole-3-acetate, Inosine, Isobutyrate, Isocaproate, Isocitrate, Isoleucine, Isopropanol, Isovalerate, Kynurenate, Kynurenine, Lactate, Lactose, Leucine, Levulinate, Lysine, Malate, Maleate, Malonate, Mannitol, Mannose, Methanol, Methionine, Methylamine, Methylguanidine, Methylmalonate, Methylsuccinate, N,N-Dimethylformamide, N,N-Dimethylglycine, N-Acetylaspartate, N-Acetylglutamate, N-Acetylglycine, N-Carbamoyl-β-alanine, N-Carbamoylaspartate, N-Isovaleroylglycine, NAD+, Niacinamide, Nicotinate, O-Acetylcarnitine, O-Phosphocholine, O-Phosphoethanolamine, O-Phosphoserine, Ornithine, Oxalacetate, Oxypurinol, Pantothenate, Phenol, Phenylacetate, Phenylacetylglycine, Phenylalanine, Pimelate, Proline, Propionate, Propylene glycol, Protocatechuate, Pyridoxine, Pyroglutamate, Pyruvate, Quinolinate, Riboflavin, Ribose, S-Adenosylhomocysteine, S-Sulfocysteine, Salicylate, Salicylurate, Sarcosine, Serine, Suberate, Succinate, Succinylacetone, Sucrose, Tartrate, Taurine, Theophylline, Threonate, Threonine, Thymine, Thymol, Tiglylglycine, Trigonelline, Trimethylamine, Trimethylamine N-oxide, Tryptophan, Tyramine, Tyrosine, Uracil, Urea, Uridine, Urocanate, Valerate, Valine, Valproate, Vanillate, Xanthine, Xanthosine, Xylose, cis-Aconitate, myo-Inositol, o-Cresol, p-Cresol, trans-4-Hydroxy-L-proline, trans-Aconitate, β-Alanine, π-Methylhistidine, τ-Methylhistidine

RTI INTERNATIONAL

Chenomx Exercise

- Save the folder called “Chenomx_Tutorial.zip into your computer
  - Sample files
  - Chenomx NMR Suite Tutorial.pdf
- We will use Processor and Profiler in the exercise
- Processor
  - Sample.fid
- Profiler
  - Basic Start, Basic End
  - Advanced Start, Advanced End
  - Batch fitting

ACKNOWLEDGEMENTS

Director RTI RCMRC
Susan Sumner, PhD

Program Coordinator
Jason Burgess, PhD

NIH Scientific Officer
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